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Plagiorchis muris infection in *Apodemus agrarius* from northern Gyeonggi-do (Province) near the demilitarized zone

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Abstract: The small intestines of 6 species of rodents and 1 species of insectivore were examined seasonally for *Plagiorchis muris* infection in 3 different localities in northern Gyeonggi-do (Province), near the demilitarized zone (DMZ). A total of 1,496 animals, including 1,366 *Apodemus agrarius*, 54 *Crocidura lasiura* (insectivore), 32 *Mus musculus*, 28 *Micronytus fortis*, 9 *Eothenomys regulus*, 6 *Micronys minutus*, and 3 *Cricetulus triton*, were live-trapped at Yeoncheon-gun (n = 351), Paju-shi (804) and Pocheon-gun (343) at 3-mo intervals from December 2004 to September 2005. A total of 1,647 *P. muris* were collected from 72 (5.3%) *A. agrarius*. The infection rate was the highest in Pocheon-gun (8.2%), followed by Yeoncheon-gun (5.0%) and Paju-shi (4.2%). A higher infection rate was observed in *A. agrarius* captured during September (19.4%) than those captured during December (3.0%), June (2.6%), or April (0%). However, the worm burden was the highest in June (av. 32.1/animal), followed by September (24.7), December (4.0), and April (0). None of the other animal species were found infected with *P. muris*. The results reveal that *A. agrarius* is a natural definitive host for *P. muris*, and infection rates and worm burdens vary seasonally and geographically.

Key words: *Plagiorchis muris*, wild rodent, *Apodemus agrarius*, prevalence, worm burden, Gyeonggi-do (Province)

Plagiorchis muris (Digenea: Plagiorchiidae) is a small (2.9-3.0 mm in size) intestinal fluke found in various mammals that feed on aquatic insects (Radomyos et al., 1989). Several human infections have been recorded (Asada et al, 1962; Radomyos et al., 1989; Hong et al., 1996). Metacercariae of *P. muris* are found in aquatic snails *Lymnaea ollula*, freshwater fish, mosquito larvae, and dragonfly larvae and adults (Hong et al., 1996, 1999). Dragonflies infected with *P. muris* metacercariae were distributed throughout the Republic of Korea, even though the infection rate and

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Table 1. Infection rates of *Plagiorchis muris* in *Apodemus agrarius* caught from 3 localities in northern Gyeonggi-do (Province), Republic of Korea

Area surveyed	No. of *A. agrarius* examined	No. positive (%)	No. of worms collected		
			Total	Mean ± SD	Range
Paju-shi	753	32 (4.2)	765	23.9 ± 51.7	1-263
Yeoncheon-gun	321	16 (5.0)	372	23.3 ± 36.3	1-111
Pocheon-gun	292	24 (8.2)	510	21.4 ± 42.8	1-186
Total	1,366	72 (5.3)	1,647	22.9 ± 45.5	1-263

metacercarial density varied according to geographical locality and dragonfly species (Hong et al., 1998, 1999). The patient infected with *P. muris* was a resident of Hamyang-gun, Gyeongsangnam-do (Province), where various freshwater fish and dragonflies were found infected with *P. muris* metacercariae (Hong et al., 1996, 1999).

In sylvatic environments, adult worms have been found in various species of mammals. Feral cats purchased in Busan had *P. muris* infections (Sohn and Chai, 2005). Commensal rodents (e.g., *Rattus norvegicus*), caught from Hadong-gun (Gyeongsangnam-do), Yongin-shi (Gyeonggi-do), and Yangyang-gun (Gangwon-do), were found infected with small numbers of *P. muris* (Seo et al., 1981; Lee et al., 1990). In a survey of sylvatic rodents caught from northern areas of the Republic of Korea (Seo et al., 1964), 5 of 6 (83.3%) *Apodemus agrarius* (the striped field mouse) were infected with *P. muris*, and the other rodent species infected was *Rattus rattus*. None (0/357) of *R. norvegicus* were found infected. Therefore, it is strongly suggested that sylvatic rodents, in particular *A. agrarius*, are an important reservoir for *P. muris* in northern areas of the Republic of Korea.

Numerous military field training sites are located along the northern boundary of Gyeonggi-do near the demilitarized zone (DMZ), where civilians are prohibited entry. Along the training sites perimeter and hills, where military activities are limited, the natural ecology is well preserved. Infection of animals with *P. muris* has never been reported from these areas. This study was initiated to determine the seasonal and geographical infection rates of *P. muris* among small mammals captured from Paju-shi, Yeoncheon-gun and Pocheon-gun (Gyeonggi-do).

A total of 1,496 small mammals including 6 rodent and 1 insectivore species, i.e., *A. agrarius* (n = 1,366), *Crocidura lasiura* (54) (insectivore), *Mus musculus* (32), *Microtus fortis* (28), *Eothenomys regulus* (9), *Micronys minutus* (6) and *Cricetulus triton* (3), were live-captured using Sherman traps (3 x 3.5 x 9″ folding traps; H.B. Sherman, Tallahassee, Florida, USA) baited with peanut butter between 2 saltine crackers. These small mammals were euthanized in accordance with an approved animal use protocol under biosafety level 3 (BSL-3) laboratory conditions. Gastrointestinal organs, including the stomach to the end of the rectum, were removed and preserved in 70% alcohol until examined. Seasonal infection rates were determined quarterly from December 2004 through September 2005. Preserved gastrointestinal organs were opened in a Petri dish containing distilled water and all helminths were collected from their internal contents under a dissecting microscope (x 10). For species identification, worms similar to *Plagiorchis* were examined morphologically under a light microscope (x 40-100). For statistical analyses of seasonal and geographical infection rates and worm burdens, the chi-square test and Fisher's exact test were used.

A total of 1,647 *P. muris* were collected from 72 (5.3%) *A. agrarius* (Table 1). The other 5 species of rodents and 1 insectivore species were not found infected with *P. muris*. Among the 3 localities, the infection rate of *A. agrarius* was the highest in Pocheon-gun, followed by Yeoncheon-gun and Paju-shi (Fig. 1A). Differences in infection rates of *A. agrarius* between Pocheon-gun and Paju-shi were significant ($P = 0.011$). The worm burden was the highest in

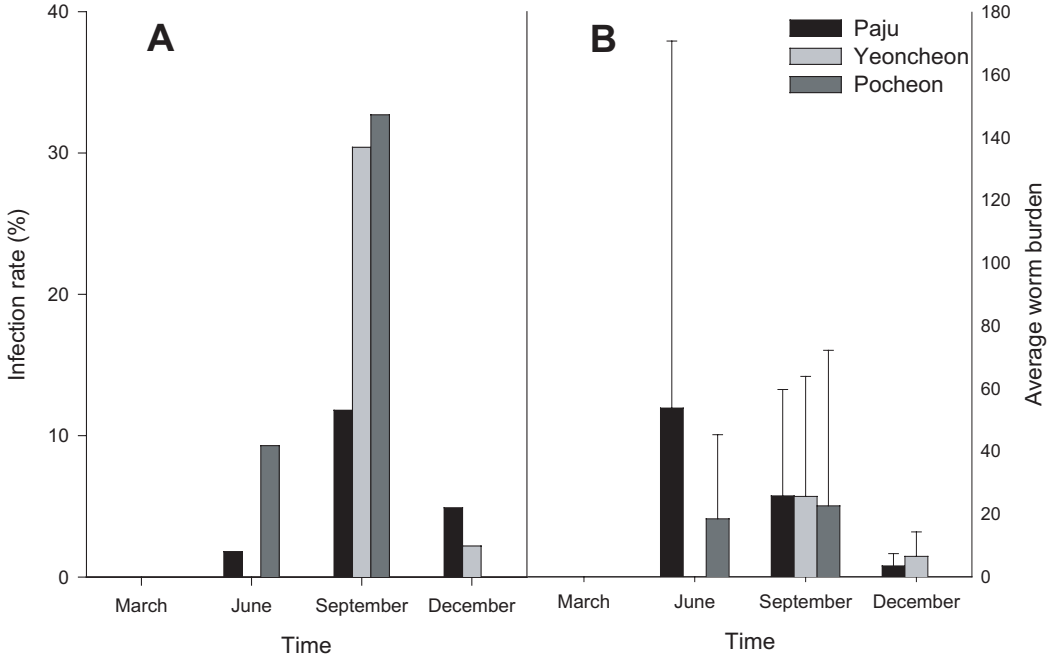

Fig. 1. Seasonal variation of infection rates of *Apodemus agrarius* **(A)** with *Plagiorchis muris* and worm burdens **(B)** in 3 different areas of Gyeonggi-do (n = 255 in March; 495 in June, 247 in September, and 369 in December.). * One *Apodemus agrarius* captured in June was infected with 263 worms, greatly increasing the overall average worm burden for that period and collection site.

Paju-shi, followed by Yeoncheon-gun and Pocheon-gun, but was not significantly different (Fig. 1B). Seasonal infection rates were variable; the infection rate was the highest in September (autumn), i.e., 48/247 (19.4%), followed by December (winter) 11/369 (3.0%) and June (summer) 13/495 (2.6%) (Fig. 1A). No *P. muris* was found in March (spring), i.e., 0/255 (0%). The worm burden was the highest in June (32.2 ± 72.8), followed by September (24.8 ± 40.1) and December (4.1 ± 4.5) (Fig. 1B). Other helminths collected during this same period will be published separately. The data on *Neodiplostomum seoulense* will be published (Chai et al., 2007) next to this paper.

During this study, *P. muris* worms were collected in only 1 rodent species, *A. agrarius*, and no *P. muris* was observed in other species of small mammals. In a previous study, 33 *P. muris* were collected from 5 *A. agrarius* and 1 *R. rattus* captured at Cheolwon-gun and Gumhwa-gun (Gangwon-do), near the present study area (Seo et al., 1964). Worm burdens (1,647 worms/72 *A. agrarius*) among *A. agrarius* infected with *P. muris* were higher than those of previous studies in commensal rodents (2 worms/2 rats) (Seo et al., 1981) and sylvatic rodents (33 worms/5 *A. agrarius* and 1 *R. rattus*) (Seo et al., 1964). Commensal rodents were not collected from the present study sites. Rodent surveys near villages, where relatively large populations of commensal rodents are found, will provide information about their role in maintenance and distribution of *P. muris*.

Based on our studies, *A. agrarius* is proved to be an important host for *P. muris* in sylvatic environments. In Japan, *A. agrarius* was also identified as a natural definitive host for *P. muris* (Ito and Itagaki, 2003). Relatively large populations of *A. agrarius* are found in tall grass and crawling vegetation habitats, often associated with water. Similar habitats along roadsides, streams, and civilian communities should be surveyed to determine the role of *A. agrarius* and other small mammals in maintenance and distribution of *P. muris* in these environments.

Infection rates of *P. muris* varied seasonally in *A. agrarius*. In all surveyed areas, infection rates were the highest in these striped field mice caught in

September. Since many insect larvae develop to adults in autumn, wild animals may have more opportunities to consume larval and adult insects that may be infected with *P. muris* metacercariae. In March, few insects are found because most adult insects do not survive the cold winter along the DMZ. Few worms were found in rodents that had been infected in previous seasons, since laboratory studies demonstrated that most infected worms (96%) in albino rates moved from the jejunum to ileum and expelled within day 28 post-infection (Hong et al., 1998). The average worm burden was the highest in *A. agrarius* trapped during June, in part due to one rodent infected with 263 worms. Excluding the data from this rodent, during the fall period, both infection rates and worm burdens were the highest.

Based on locality, *P. muris* infections were variable. Rodent infection rates in Pocheon (8.2%) were much higher than those in Paju (4.2%) and Yeoncheon (5.0%). During the March trapping period, *P. muris*-infected rodents were not found in all 3 areas surveyed, while in June and December, *P. muris* was not observed in *A. agrarius* captured at Yeoncheon and Pocheon. Whereas there were no remarkable differences in worm burdens of *P. muris* in rodents according to locality, seasonal differences were observed. The average worm burden in infected rodents was the highest in June in Paju, due to one rodent with a very high worm burden (263 worms), but in Yeoncheon and Pocheon, those were the highest in September. This is mostly likely due to an accumulation of ingested metacercariae by eating infected insects over the late summer and early fall period.

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